

**Directions:** Answer the following questions in complete sentences, and make sure your figures are formatted in the document correctly. You may work with your partner, but each person must submit their own copy of this assignment. Question 6 **must** be written in your own words. Review assignment 3 for more information on any of the questions below.

**QUESTION:** Is atropine an effective medication to treat heart failure in *L. catesbeianus*?

Our results suggest that atropine may not be an effective medication in the treatment of heart failure in *L. catesbeianus*, although it may offer rescuing effects in regards to pulse frequency.

1) **SIGNIFICANCE:** What was the purpose of this study? Why was the question important, and what do we hope to gain from the results?

The ability to “rescue” the heart from arrhythmia is of critical importance to medical wellbeing. Irregular cardiac activity may account for 80% of all cardiac arrest mortalities<sup>(1)</sup>. In this experiment, we artificially replicated a low heart rate within *L. catesbeianus* in order to test whether the introduction of atropine could restore normal cardiac rhythm. Our hope was to demonstrate the effectiveness of atropine as a cardiac rescue-agent.

Mehra, Rahul. "Global public health problem of sudden cardiac death." *Journal of electrocardiology* 40.6 (2007): S118-S122.2)

2) **HYPOTHESIS:** What is your hypothesis for this study?

Administration of 10mM atropine will reverse the effects of 5mM acetylcholine within the heart of *L. catesbeianus* (pulse frequency, pulse amplitude, and pulse duration will all recover to baseline levels).

**Do you think acetylcholine will increase, decrease, or cause no change in the heart rate (frequency), force duration, or force amplitude?**

Addition of acetylcholine to the bullfrog heart will result in a significant decrease in heart rate frequency, a significant decrease in force duration, and a significant decrease in force amplitude.

**Do you think atropine will increase, decrease, or cause no change in the heart rate (frequency), force duration, or force amplitude?**

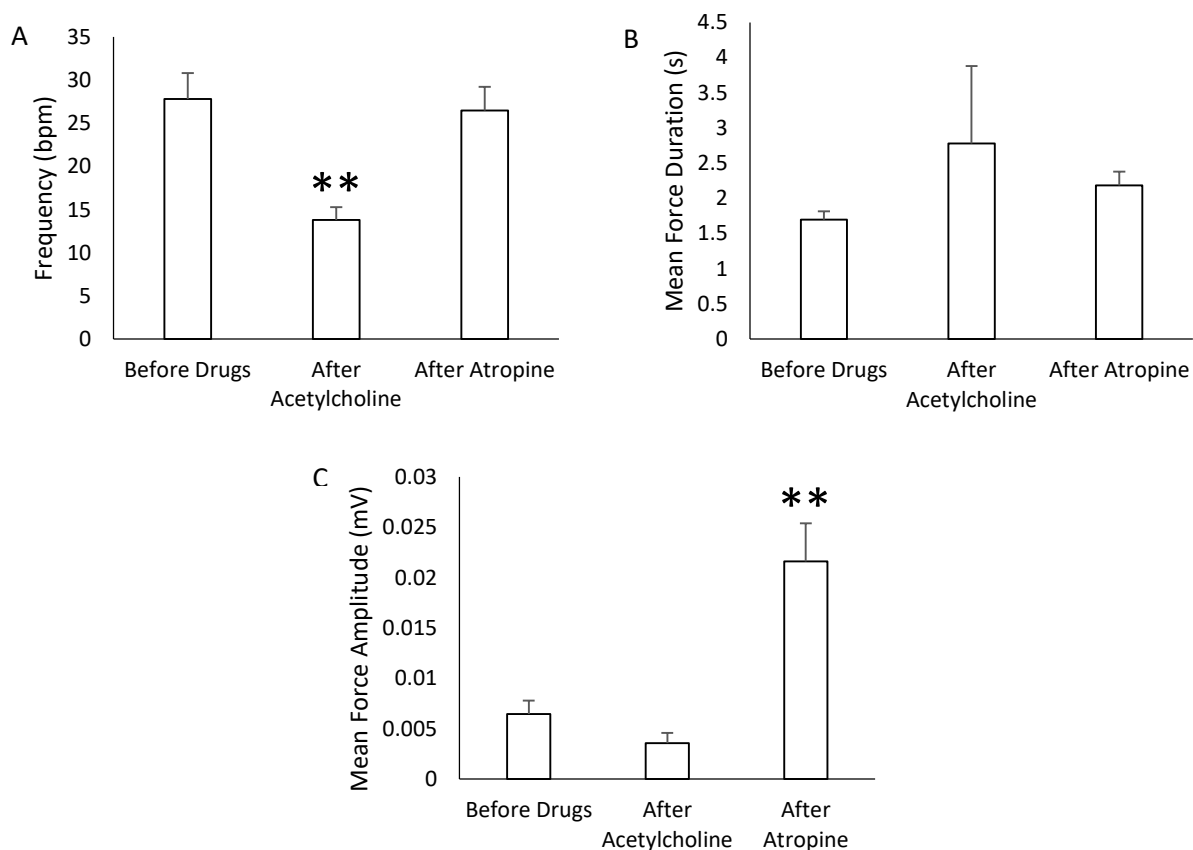
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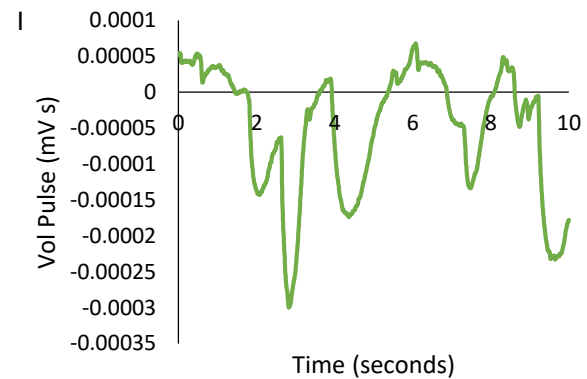
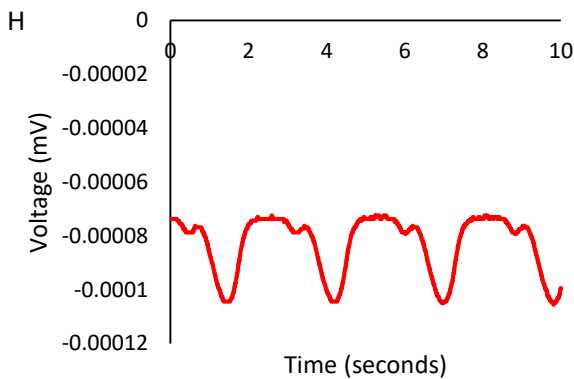
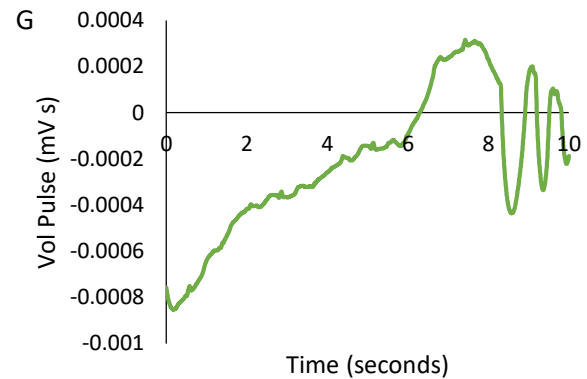
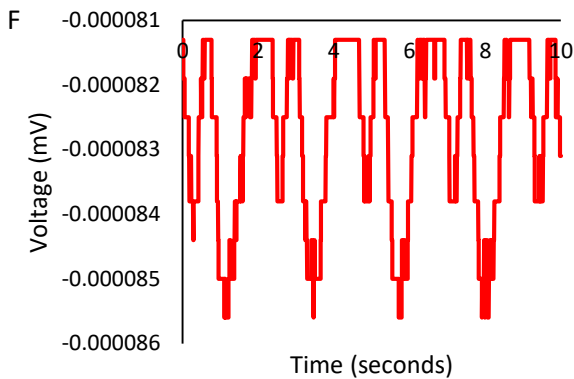
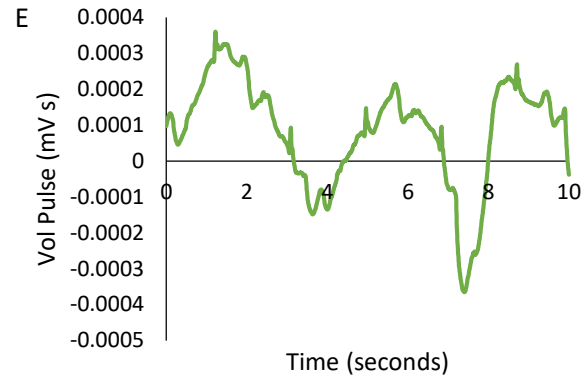
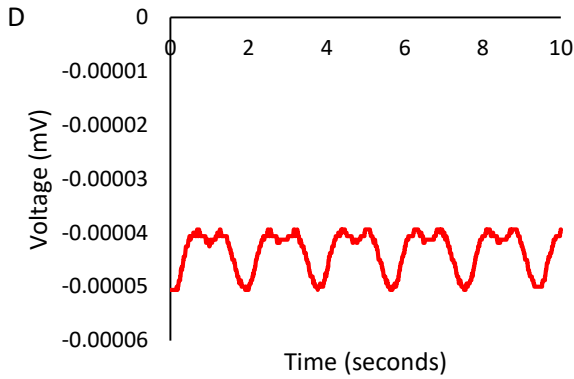
3) **FIGURES:** include a graph representing the calculated data, a chart representing the raw data that complements the data depicted in the graph, and a figure legend for each of the following:

Figure 1: Before addition of drugs, after acetylcholine, and after atropine

Charts should represent the raw signals for the Force (channel 1) and the ECG (channel 2), and should represent the data in the graph. Make your charts with respect to the frequency data, as all other parameters can be inferred from this data. If your data does not resemble the class data, you do not need to include these charts; you will, however, need to explain this in question 6 and explain why you think your data differed from the class data. If results are not significant, you should include charts that are representative of your own individual data. Finally, you may not use the same chart for any figure. Although the data will look nearly identical, it is bad practice to repeat images, even if it shows the same thing.

**Figure 1: Cardiac physiology of the bullfrog before addition of drugs, after acetylcholine, and after atropine. A.** Comparison of the mean frequency of ECG signals, **B.** force duration, and **C.** force amplitude. **D – E.** Representative ECG and force signal data respectively before addition of drugs, **F – G.** after acetylcholine, and **H – I.** after atropine. Bars = mean  $\pm$  SEM. Student t-test, \* $p < .05$ ; \*\* $p < .01$ . A,B,C:  $n = 15$ .





#### 4) RESULTS: a) Describe any physical changes observed during the experiment.

##### While recording after the addition of acetylcholine:

Small change at first (decreased amplitude)

then gradual lowering of beat amplitude and duration between beats.

##### While recording after the addition of atropine:

Increases heart beat amplitude visible upon first drops. Duration pretty much the same.

**b) Write a simple statement describing the results for each graph in question 3 (3 statements). Statements can be written in the form:**

“There was a/no significant effect of *manipulation* on *measured outcome*, T-test:  $p = p\text{-value}$  or Repeated Measures ANOVA:  $F(df_B, df_W) = F \text{ value}, p = p\text{-value}.$ ”

**Figure 1 statement:**

There was a **significant** effect of *ECG frequency*, Repeated Measures ANOVA Sphericity assumed:  $F(2,28) = 10.477, p = .000.$

There was **no** significant effect of *force duration* Repeated Measures ANOVA Greenhouse-Geisser:  $F(2,26) = .812, p = .389.$

There was a **significant** effect of *force amplitude*, Repeated Measures ANOVA Greenhouse-Geisser:  $F(2,28) = 21.866, p = .000.$

**5) CONCLUSIONS:** Did the data collected support or refute your hypotheses developed in questions 2? Refer to the figures above to justify your answers.

**Overall hypothesis**

Pulse frequency was the only measured variable that was rescued, in the sense that it demonstratively returned to the baseline levels. Pulse duration did not show a significant increase with acetylcholine, so atropine could not conclusively perform any rescue action. Finally, pulse amplitude was at approximately 335% of the baseline levels following atropine, and therefore was “over-rescued”—a potentially hazardous medical effect.

**Acetylcholine hypothesis**

The collected data supports our hypothesis that when acetylcholine is added, there will be a significant decrease in the frequency of electrical signals of the class (Fig. 1.A). However, we must reject our hypothesis that acetylcholine will decrease the force duration, as no significant change was observed (Fig. 1.B). Finally, we did not observe a significant decrease in force amplitude following addition of acetylcholine (Fig. 1.C). The representative ECG measurements add support for these observations (Fig. 1.F and 1.G).

**Atropine hypothesis**

The collected data supports our hypothesis that when atropine is added, there will be a significant increase in the frequency of electrical signals relative to the “After Acetylcholine” frequency (Fig. 1.A). However, we must reject our hypothesis that atropine will decrease the force duration, as no significant change was observed (Fig. 1.B). Finally, we observed an increase in force amplitude following addition of atropine, supporting our hypothesis (Fig. 1.C). The representative ECG measurements add support for these observations (Fig. 1.H and 1.I).

6) **DISCUSSION:** Combine questions 1 and 5 to make a complete discussion section, and refer to question 3 and 4 to connect the data to the larger picture (do not include p-values). This section should be no longer than 500 words. Make sure you summarize the question and most important findings at the beginning, describe the patterns/relationships data shows, and connect it to the broader context at the end.

Anything that is not interesting does not need to be discussed in detail, but you do need to mention that you have the data and why it is not interesting (not significant), as well as discuss charts that were left out due to differences in your data compared to the class. Combine results/information to make your discussion as concise as possible. Finally, make sure you include any potential problems that may have skewed the results, and explain ways to control for this in the future.

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The ability to “rescue” the heart from arrhythmia is of critical importance to medical wellbeing. Irregular cardiac activity may account for 80% of all cardiac arrest mortalities<sup>(1)</sup>. In this experiment, we administered 10mM acetylcholine to the heart of the American Bullfrog (*L. catesbeianus*) in order to mimic the effects of heart failure. Following a given amount of time, we then administered 30mM atropine to test whether the atropine could restore normal cardiac rhythm. Our hope was to demonstrate the effectiveness of atropine as a cardiac rescue-agent. Overall, we tested 15 bullfrogs in this manner. Measured variables include pulse frequency, pulse duration, and pulse amplitude.

Overall, pulse frequency was the only measured variable that was rescued, in the sense that it demonstratively returned to the baseline levels. Pulse duration did not show a significant increase with acetylcholine, so atropine could not conclusively perform any rescue action. Finally, pulse amplitude was at approximately 335% of the baseline levels following atropine, and therefore was “over-rescued”—a potentially hazardous medical effect.

In regards to acetylcholine, the collected data supports our hypothesis that when acetylcholine is added, there will be a significant decrease in the frequency of electrical signals of the class (Fig. 1.A). However, we must reject our hypothesis that acetylcholine will decrease the force duration, as no significant change was observed (Fig. 1.B). Finally, we did not observe a significant decrease in force amplitude following addition of acetylcholine (Fig. 1.C). The representative ECG measurements add support for these observations (Fig. 1.F and 1.G).

In regards to atropine, the collected data supports our hypothesis that when atropine is added, there will be a significant increase in the frequency of electrical signals relative to the “After Acetylcholine” frequency (Fig. 1.A). However, we must reject our hypothesis that atropine will decrease the force duration, as no significant change was observed (Fig. 1.B). Finally, we observed an increase in force amplitude following addition of atropine, supporting our hypothesis (Fig. 1.C). The representative ECG measurements add support for these observations (Fig. 1.H and 1.I).

Our results suggest that atropine may not be an effective medication in the treatment of heart failure in *L. catesbeianus*, although it may offer rescuing effects in regards to pulse frequency. The lack of any significant rescue in pulse duration and the “over-rescue” effect on pulse amplitude does not support the medical effectiveness of atropine as a drug that could reverse the symptoms of heart-failure. However, the rescue of pulse frequency in our organisms makes it a promising focus for future research, especially in regards to cardiac health and overall physiological wellbeing.